EVALUATION OF ANTI-SEIZURE ACTIVITY OF *SIDA RHOMBIFOLIA* ALONE AND IN COMBINATION WITH ANTI-SEIZURE DRUGS IN SWISS ALBINO MICE

Pharmacology

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ABSTRACT

Background- Anti-seizure activity of *Sida Rhombifolia* in low (300mg/kg) and high doses (600mg/kg) and its combination with Phenytoin (50mg/kg) and Sodium valproate (300mg/kg) were studied in chronic model (14 days) of Maximal Electroshock Seizure (MES) and Pentylenetetrazole (PTZ) induced seizure respectively.

Methods- Anti-seizure activity of *Sida Rhombifolia* on generalized tonic-clonic seizure and absence seizure was evaluated using standard maximal electroshock seizure method and pentylenetetrazole test.

Results- In MES induced seizures *Sida Rhombifolia* in high dose prevented both tonic extension and tonic flexion. In PTZ induced seizures *Sida Rhombifolia* in high dose significantly ($p < 0.001$) increase the latency of onset of seizures and both high and low dose *Sida Rhombifolia* significantly decrease the duration of seizure ($p < 0.01$) as compared to vehicle control, however, there was no significant effect on onset of 1st myoclonic jerk ($p > 0.05$). The addition of *Sida Rhombifolia* to the sub-therapeutic dose of sodium valproate and phenytoin showed a synergistic effect.

Conclusion- Inhibition of seizure by *Sida Rhombifolia* could be due to the presence of flavonoids that acts as a partial positive allosteric modulator at GABAA (γ -aminobutyric acid) receptors, penetrates the blood-brain barrier and possess the anti-convulsant activity and also because of its antioxidant property. *Sida Rhombifolia* can be effective in generalized tonic-clonic seizures alone and as add on with sodium valproate for absence seizures.

KEYWORDS

Sida Rhombifolia, Seizures, Pentylenetetrazole (PTZ), MES (maximal electroshock), Phenytoin, Sodium Valproate.

INTRODUCTION-

A seizure is a transient alteration of behavior due to the disordered synchronous and rhythmic firing of populations of brain neurons. Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. Seizures are characterized by brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized) and are sometimes accompanied by loss of consciousness and control of bowel or bladder function (Brunton LL, 2016, p. 314). Approximately 50 million people currently live with epilepsy worldwide, of that 80% reside in developing countries. The overall prevalence in INDIA is 5.59-10 per 1000 (Santhosh NS et al., 2014).

Management of epilepsy requires prompt attention and constant treatment. About 75-80% of patients achieve control with traditional anti-epileptic drugs like phenytoin, sodium valproate, and carbamazepine. Most of these drugs have a restricted therapeutic margin and require therapeutic drug monitoring. Around 20-25% of patients develop therapeutic failure with these drugs. Moreover, due to adverse reactions, drug interactions, and long term treatment, compliance is a major problem with anti-epileptic treatment (Anovadiya A et al., 2012).

Both Phenytoin and Sodium valproate produce dose-dependent adverse drug reactions which can also be reduced by using the sub-therapeutic dose of drug synergistically with the new compounds having the same efficacy, so all this stimulates further research to find newer drugs and new management modalities. (Sander JW et al., 2009) and (Wu Fu et al., 2013).

Sida Rhombifolia (L.) is widely known in Ayurvedic medicine for its anti-inflammatory and anti-oxidant effects. *Sida Rhombifolia* (L.) states to possess an anti-epileptic effect (Duke J.A., 2002). Other indications are depression, mental instability, irritability, mood enhancer, hemiplegia, Parkinson's, whooping cough, rheumatism, herpes, warts, hemorrhoids, bronchitis, and cough. Though one study has been done in the past for *Sida Rhombifolia* on the anti-seizure effect that was an acute study (Sharma V et al., 2013). This study was planned to evaluate its anti-seizure effect in low and high dose and its add on effect with gold standard antiepileptic drugs phenytoin and sodium valproate in therapeutic and sub-therapeutic doses via the oral

route of administration in two different chronic models in Swiss albino mice.

Methodology-

All the experiments were performed after taking prior permission from the Institutional Animal Ethics Committee (IAEC). Swiss albino mice (*mus musculus*), three-four months of age (26 to 34 g) of either sex were procured from the central animal house of the institute. They were housed in standard transparent polypropylene cages (25cm × 19cm × 13cm) with wheat husk bedding kept under controlled room temperature and humidity ($26 \pm 3^\circ\text{C}$; $40 \pm 5\%$) in a 12 hours light-dark cycle. The animals were kept on standard laboratory diet and water *ad libitum*. All the animals were acclimatized to the laboratory conditions at least one hour prior to the experiments. Food was withdrawn 12 hrs before the experiments. The animal handling was performed as per the Good Laboratory Practice (GLP) guidelines.

Drugs and chemicals-

Free samples of *Sida Rhombifolia* (L.) (Tulsi Amrit Private limited, Indore with batch no.-08A11601), analytical grade Sodium Valproate (Pure and Cure Private Limited, Haridwar Uttarakhand), Phenytoin and Pentylenetetrazole (Sigma Aldrich, Bangalore, India) and Gum Acacia (Fischer Scientific, Mumbai, India) were used in the study. The plant material was authenticated by the Department of Botany, Bhavnagar University, Bhavnagar, Gujarat.

Study design-

The anti-seizure activity of *Sida Rhombifolia* and its combination with phenytoin and sodium valproate was evaluated by maximal electroshock (MES) and pentylenetetrazole (PTZ) induced seizures respectively after chronic dosing (14 days) in mice. Both procedures were started 60 min after oral treatment on day 14 with the test compound or the vehicle. Recordings were done for both the experiments and were later analyzed by a rater who was blinded to the treatment. Each group contained 8 animals and animals were randomized using Rando software.

MES induced seizure-

MES test was performed as cataloged by Giardina and Gasior 2009. Digital Electroconvulsimeter (Orchid Scientific India) was used along with ear electrodes to deliver the shock. The intensity of the

stimulus given was 122 mA for 0.2s. Video recording of all animals was done. The recordings were later evaluated by a rater who was blinded to the treatment given. Parameters like total time of tonic flexion, tonic extension, and clonic phase duration were calculated by stopwatch. The endpoint for anticonvulsant activity in MES is the inhibition of tonic hind limb extension. Animals were divided into six groups, each group having eight animals. The route of administration was oral for all groups. Group 1 received 5% gum acacia and considered as vehicle control. Group 2 Phenytoin (50mg/kg). Group 3 and 4 received *Sida Rhombifolia* in the dose of (300 mg/kg) and (600 mg/kg) respectively. Group 5 received Phenytoin (50mg/kg) + *Sida Rhombifolia* (600mg/kg) and group 6 received Phenytoin (25mg/kg) + *Sida Rhombifolia* (600mg/kg).

PTZ induced seizure-

On day 14, 60 minutes after the last dose, PTZ was administered intraperitoneally (i.p.) in the dose of 75 mg/kg and animals were stowed into an individual plastic cage for observation lasting 60 minutes and video recording was done for initial 15 minutes and parameters like time of onset of first myoclonic jerk, time of onset of tonic-clonic seizures, duration of seizures and intensity of seizures by using Racine's seizure intensity scale (Luttjohann A et al., 2009) and % protection against mortality were calculated. Delay in the onset of a first myoclonic jerk, delay in onset or prevention of tonic-clonic convulsion and mortality was taken as the measure of antiepileptic activity. Animals were divided into six groups, each group having eight animals. Group 7 received vehicle Control (distilled water). Group 8 Sodium Valproate (300mg/kg). Group 9 and 10 *Sida Rhombifolia* (300mg/kg) and (600mg/kg). Group 11 Sodium Valproate (300mg/kg) + *Sida Rhombifolia* (600mg/kg). Group 12 Sodium Valproate (150mg/kg) + *Sida Rhombifolia* (600mg/kg).

Statistical test-

Data are illustrated as the Mean $\pm$ Standard error of the mean (SEM). Statistical analysis for MES and PTZ induced seizures was performed using Graph Pad prism Version 6.0h (Demo Version) statistical software. For parametric data ANOVA (Analysis of variance) followed by Tukey Kramer post-test and for non-parametric data Kruskal Wallis followed by Dunn's multiple comparison tests. A value of $p < 0.05$ was considered statistically significant.

Results-

MES test- *Sida Rhombifolia* at high dose (600mg/kg) abolished total hind limb extension as well as tonic flexion phase but failed to do that in low dose *Sida Rhombifolia* (300mg/kg). Tonic extension and tonic flexion were prevented in Phenytoin (50mg/kg) and add on groups as well. The clonic phase was present in all the groups. There was no significant difference ($p > 0.05$) in phenytoin (50mg/kg) as compared to vehicle control. A significant reduction in the duration of clonic phase was seen in both the add on groups as compared to vehicle control ($p < 0.001$).

Table 2 Effect of vehicle and test drug on Pentylene tetrazole induced seizure in Swiss albino mice. (n=8).

Treatment groups	Dose (mg/kg) Oral	1st myoclonic jerk (secs) (mean $\pm$ SEM)	Latency of onset of seizures (secs) (mean $\pm$ SEM)	Duration of seizures (secs) mean $\pm$ SEM	% Protection	Seizure intensity stage mean $\pm$ SEM
Group- 1 (Vehicle control)	2.5ml/kg	35.75 $\pm$ 4.02	58.37 $\pm$ 3.90	19.25 $\pm$ 0.97	0%	6 $\pm$ 0.00
Group- 2 Sodium Valproate	300	309.87 $\pm$ 24.97*	Prevented	Prevented	100%	1.25 $\pm$ 0.16*
Group- 3 <i>Sida Rhombifolia</i>	300	58.12 $\pm$ 4.34	113.75 $\pm$ 11.59	9.25 $\pm$ 0.75*	0%	6 $\pm$ 0.00
Group- 4 <i>Sida rhombifolia</i>	600	97.75 $\pm$ 5.97	292.37 $\pm$ 40.4*	8.37 $\pm$ 0.99*	75%	5 $\pm$ 0.00
Group-5 Sodium Valproate (300mg/kg) + High dose <i>Sida Rhombifolia</i>	300+600	358.87 $\pm$ 27.07*	Prevented	Prevented	100%	1 $\pm$ 0.00*
Group-6 Sodium Valproate(150mg/kg) + High dose <i>Sida Rhombifolia</i>	150+600	143.12 $\pm$ 8.85*	Prevented	Prevented	100%	3 $\pm$ 0.00*

* means $p < 0.05$ statistically significant as compared to vehicle vehicle control. Statistical analysis of data is done by Kruskal –Wallis test followed by Dunn's multiple comparison test for all variables. p -value < 0.05 was considered significant. % protection is calculated as survived/used multiply by 100.

DISCUSSION-

Maximal electroshock (MES) seizures in cats, led to the discovery of phenytoin for generalized tonic-clonic seizure. The subcutaneous

Table 1 Effect of vehicle and test drug on MES induced seizure in Swiss albino mice. (n=8).

Treatment groups	Dose (mg/kg) Oral	Tonic flexion (secs) (mean $\pm$ SEM)	Tonic extension (secs) (mean $\pm$ SEM)	Clonic phase (secs) mean $\pm$ SEM
Group- 1 (Vehicle control)	2.5ml/kg	1.84 $\pm$ 0.177	14.18 $\pm$ 0.83	11.71 $\pm$ 0.89
Group- 2 Phenytoin	50	Prevented	Prevented	8.65 $\pm$ 0.75s
Group- 3 <i>Sida Rhombifolia</i>	300	1.28 $\pm$ 0.15	10.81 $\pm$ 0.89*	8.01 $\pm$ 0.41
Group- 4 <i>Sida Rhombifolia</i>	600	Prevented	Prevented	7.80 $\pm$ 0.66
Group-5 Phenytoin 50mg/kg + High dose <i>Sida Rhombifolia</i>	50+600	Prevented	Prevented	4.28 $\pm$ 0.16*
Group-6 Phenytoin (25mg/kg) + High dose <i>Sida Rhombifolia</i>	25+600	Prevented	Prevented	5.65 $\pm$ 0.26*

* means $p < 0.05$ statistically significant as compared to vehicle vehicle control. Statistical analysis of data is done by Kruskal –Wallis test followed by Dunn's multiple comparison test for tonic flexion. For tonic extension data analysis is done by one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test. For clonic phase data analysis is done by Kruskal- Wallis test followed by Dunn's multiple comparison test.

PTZ test- Myoclonic jerks were produced in all the groups. However significant reduction in duration of onset of 1st myoclonic jerk was seen in Sodium Valproate ($p < 0.001$) and add on groups of therapeutic dose of Sodium valproate (300mg/kg) + high dose *Sida Rhombifolia* (600mg/kg) ($p < 0.001$) and sub-therapeutic dose of Sodium valproate (150mg/kg) + high dose *Sida Rhombifolia* (600mg/kg) ($p < 0.05$) as compared to vehicle control. Seizures were not observed after treatment in active control i.e. Sodium valproate group as well as both the add on groups. However, there was an increase in latency of onset of a seizure in high dose *Sida Rhombifolia* (600mg/kg) as compared to vehicle control i.e. distilled water ($p < 0.001$). Low dose *Sida Rhombifolia* and high dose *Sida Rhombifolia* showed a significant decrease in duration of seizures as compared to vehicle control ($p < 0.01$).

(s.c.) pentylenetetrazole (PTZ) test (for absence seizure) used in mice and revealed the anticonvulsant properties of trimethadione. These two models have been used for several decades and still remain the mainstay for two key preclinical tests for random screening aimed for the detection of new AEDs (anti-epileptic drugs).

Maximal electroshock seizure test helps the testing of drugs acting on Na⁺ channels (e.g., carbamazepine, phenytoin), the majority of standard and newly developed anticonvulsant drugs are effective in the

MES model. Stimulation through ear-clip electrodes activates the brain stem. (Castel-Branco et al., 2009) and (Perucca et al., 2007). In the present study, *Sida Rhombifolia* at high dose (600mg/kg) abolished total hind limb extension as well as tonic flexion phase but failed to do that in low dose *Sida Rhombifolia* (300mg/kg). Phenytoin, as well as *Sida Rhombifolia*, failed to prevent clonic phase but there was significant reduction in duration of the clonic phase in both the add on groups as compared to vehicle control ($p < 0.001$). This prevention of tonic extension and flexion in the MES test by the high dose of *Sida Rhombifolia* may be due to the presence of flavonoids which have got anticonvulsant and CNS depressant activity (Sharma V et al., 2013). Flavonoids act as a partial positive allosteric modulator at GABA_A receptors penetrates the blood-brain barrier and encounters the anticonvulsant activity. Other plants of the same *Sida* family-like *Sida Tiaggi* and *Sida Cordifolia* also have an effect on the benzodiazepine site of GABA receptors in a mouse's brain. (Abat JK et al., 2017)

PTZ is a tetrazole derivative with a consistent effect on a large number of animal species. It acts by antagonizing the inhibitory GABAergic neurotransmission by blocking its chloride channel. PTZ produces oxidative stress to neuronal cells. The test is predictive of anticonvulsant drug activity against absence seizures (Kasthuri S et al., 2013). In the present study myoclonic jerks were produced in all the groups. However, the significant reduction in duration of onset of 1st myoclonic jerk was seen in Sodium Valproate and add on groups as compared to vehicle control. A significant increase in the duration of latency of onset of seizures was seen in high dose *Sida Rhombifolia* (600mg/kg) as compared to vehicle control ($p < 0.001$). Both high dose *Sida Rhombifolia* (600mg/kg) and low dose *Sida Rhombifolia* (300mg/kg) showed a significant reduction in duration of seizures as compared to vehicle control ($p < 0.01$). Seizure intensity staging for PTZ induced seizures i.e. for behavioral patterns during a seizure showed no significant difference in intensity stage of low dose *Sida Rhombifolia* and high dose *Sida Rhombifolia* as compared to vehicle control ($p > 0.05$). *Sida Rhombifolia* is well proven for its anti-oxidative properties; this might be the reason for its anticonvulsant mechanism in PTZ induced seizure, a lot of oxidative stress is produced by PTZ (Narendhirakannan RT et al., 2010). The oxidative stress is one of the most prominent mechanisms in the development and progression of epilepsy. The constituent in this herb that is Bala (also present in *Sida cordifolia* Linn. and *Sida retusa* Linn.) is well demonstrated for anti-oxidants. The presence of anti-oxidant prevents the possible damage of neurons occurring via repeated seizures (Nimmy VS et al., 2017).

Similar kind of results were also seen in the previous study done by Sharma V et al., 2013 that is methanolic extract of *Sida Rhombifolia* at dose 100 mg/kg, 200mg/kg and 400mg/kg significantly reduced the duration of the seizures as compared to controls (normal saline) in acute model of MES induced seizure ($p < 0.0001$) and a significant increase in the latency to clonic convulsions were observed with *Sida Rhombifolia* treated groups (100 mg/kg, 200mg/kg and 400mg/kg single dose) compared with vehicle controls (normal saline) in pentylenetetrazole induced seizures.

For MES model *Sida Rhombifolia* in high dose prevented convulsions. Adjunctive groups of therapeutic and sub-therapeutic phenytoin + high dose *Sida Rhombifolia* produce an effect similar to phenytoin. Thus a high dose of *Sida Rhombifolia* can be used alone or in conjunction with a sub-therapeutic dose of phenytoin for generalized tonic-clonic convulsions. For absence seizures, both adjunctive groups showed results similar to sodium valproate. Thus *Sida Rhombifolia* has a synergistic role with sodium valproate. The advantage of using combination of sub-therapeutic dose of phenytoin and sodium valproate and high dose *Sida Rhombifolia* will be that adverse effects that are associated with therapeutic dose of both established drugs can be reduced and also *Sida* has good safety profile so can be used as concomitant therapy also (Sundaraganapathy R et al. 2013) and (Mah SH et al., 2017).

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